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Full Length Research Paper

Association of *IL-10*-592 *A/C*, *IL-10*- 1082 *G/ A* polymorphisms with prostate cancer in North Indian population

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Prostate cancer, unlike cancers of other sites, often has a very indolent natural history. Cytokines such as interleukin 10 (*IL-10*) may play an important role in prostate cancer through inflamination. Single nucleotide polymorphism (SNP) may regulate the biosynthesis, activations, and inactivation of genes and could influence the pathogenesis of disease initiation and progression. The aim of the present study is to assess the association of the two polymorphisms of *LI-10* (592C/A and 1080*G-A*) with risk of prostate cancer. One hundred fifty prostate cancer patients, one hundred fifty Benign Prostate Hyperplasia and equal number of age matched control groups were recruited from north Indian population for this case control study. The polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were utilized to amplify the gene of interest and identify the genotypes of *IL-10* 592 and IL-101080. Statistically non significant 1.74 and 1.67 folds of increased risk of prostate cancer was observed due to the gene-gene interactions of *AC* of *IL-10*-592 and *GG* of *IL-10* 1082 and *AA* of *IL-10* -592 and *CC* of *IL-10* 1082, respectively.

Keywords: Prostate cancer: Bennie prostate hyperplasia: IL-10; polymorphisms.

INTRODUCTION

Prostate cancer is a common condition worldwide. Different geographical regions have varying incidence and mortality. Globally, prostate cancer is the sixth most common cancer, and the third most common cancer in men in developed countries. The risk of prostate cancer is increased by African-American ethnicity, increasing age, positive family history, and other factors such as diet. Nonetheless, the causes of prostate cancer are not well understood compared with other common cancers like lung and breast cancer (Kumar et al., 2004). *IL-10* is a pleiotropic cytokine with both anti-inflammatory and anti-angiogenic properties. The anti-inflammatory properties of IL-10 are hypothesized to have pro-tumorigenic potential by enabling tumor cells to escape immune surveillance. Paradoxically, IL-10 also has anti-tumorigenic properties, since in both animal and *in vitro* models; IL-10 has been shown to reduce both tumor growth and angiogenesis (Huang et al., 1996; Stearns et al., 1997; Stearns and Wang, 1998; Stearns et al., 1999). The gene encoding IL-10 is located on chromosome 1 (1q31-1q32), and many polymorphisms of the *IL-10* gene promoter have been described. Examples of polymorphisms include -1082 *A/ G*

(rs 1800896), -819 T/C (rs1800871) and -592 *A/C* (rs1800872) in the proximal region, which influence the transcription of IL-10 mRNA and the expression of IL- 10 *in vitro* (Kingo et al., 2005 ; Turner et al., 1997; Gibson et al., 2001). *IL-10-G*1082*A* polymorphism is correlated with the expression of IL-10 and accordingly affects the susceptibility to some types of tumors, such as cervical cancer (Roh et al., 2002) and prostate cancer (McCarron et al., 2002). Although the expression of IL-10 gene might be associated with the susceptibility to oesophagal scoumous cell carcinoma (Nagata et al., 2002), the correlation of *IL-10* -1082 promoter polymorphism with the risk of prostate cancer in north Indian population has not been reported so far. Some workers have suggested that as much as 75% of inter-individual variation in IL-10 expression may be due to genetic variation, although others believe that the contribution of individual SNPs – such as the best-described -1082 SNP – may be much less than this. However, at this stage the precise role of *IL-10* promoter polymorphisms – both individually and as part of defined haplotypes and mosaics – in determining IL-10 transcription and expression levels is still a subject under active investigation (Howell and Rose-Zerilli, 2007). Multiple studies have investigated the association between IL-10 expression and incidence of PCa (Wang et al., 2009 ;Kesarwani et al., 2009; McCarron et al., 2002). Polymorphisms of the *IL-10* promoter at -1082 (Eskdale and Gallagher, 1995 ;Crawley et al., 1999), and -592 (Faupel-Badger et al., 2008) reduce protein expression, and associate with an increased incidence of PCa in some reports (McCarron et al., 2002; Eskdale and Gallagher, 1995 ;Crawley et al., 1999 ;Faupel-Badger et al., 2008). In contrast, genetic variation at -592, or -1082 or the promoter haplotype ATA resulted in no association in others (Eder et al., 2007 ; Michaud et al., 2006). These discrepancies suggest that further investigation of the association of *IL-10* SNPs with PCa is acceptable. Additionally, no such studies have analyzed the impact of these polymorphisms on PCa risk in north Indian patients. Therefore, the present study aims in analyzing the association of *IL-10* 592 *C/A*, *IL- 10* 1082*G/A* polymorphisms with prostate cancer risk in north Indian population.

MATERIALS AND METHOD

Characteristics of study subjects

This study included 150 diagnosed prostate cancer cases recruited from the hospitals of post graduate institute of medical education and research, Chandigarh, all Indian Institute of Medical research, New Delhi and Government medical college hospital, Patiala, north India. 150 benign prostate hyperplasia age matched subjects and 150 health control groups from the same geographical area were also participated in this case control study. All healthy control patients underwent screening and had a normal serum prostate-specific antigen (PSA) level (< 4 ng/mL).

Genotyping of LI-10-592 C/A and IL-101080G/A

Blood samples of study subjects were collected in EDTA coated vacutinous tubes. Genomic DNA was extracted from epithelial buccal cells with sequential phenol/chloroform solution and precipitated with salt/ethanol solution (Scarel-Caminaga et al., 2004). Genotyping of the promoter region of *IL-10* -592*C/A* polymorphisms were amplified using the following primers: forward 5'-CCTAGGTCACAGTGACGTAA-3', reverse 5'-GGTGAGCACTACCTGACTAGC-3'. PCR reaction was performed in a total volume of 25µl reaction mixture containing 100ng of genomic DNA, 10X KCl buffer, 1.5mM $MgCl_2$, 0.2mM of each dNTP, 25 pm of each primer, 1 U Taq polymerase and 19µl of distilled water. The PCR condition was denaturation at 95^0C for 5 min. followed by 30 cycles of 95^0C for 45 seconds, 58^0C for 45 seconds, 72^0C for 1 min. for denaturation, annealing and extension, respectively and final extension at 72^0C for 7 min. RFLP reaction was carried out in 15µl reaction mixture containing 10µl of 412bp PCR product (Fig. 1), 1.8µl of buffer, 2 U of RSa I enzyme and 3µl of distilled water. The reaction mixture was incubated at 37 ^{o}C for 16 hours. The digestion with Rsa I site yielded 236 and 176bp products. The undigested 412bp product revealed *AA* and the digested fragment *CC* genotype. The heterozygous *AC* genotype was identified by the presence of 412, 236 and 176 bp products (Fig. 2)

The promoter region of *IL 10*- 1082 single nucleotide polymorphism (PCR-ARMS (amplified resonance mutation system)) was amplified with primers following the techniques of Langsenlehne *et al. (*2005) that generated 313 pb PCR product (Langsenlehner et al., 2005). The primer sequences are Forward 5'-TTTCCAGATATCTGAAGAAGTCCTG-3', Reverse 5'-GTAAGTTTCTGTGGCTGTAGTC-3',the *G* alleles 5'-AACACTAAGGCTTCTTTGGGTG-3' and the *A* allele with 5'-AACACTAAGGCTTCTTTGGGTA-3'. Similar PCR reaction mixture like the *IL*-592*C/A* was utilized in the amplification of *IL-10*80*G/A* polymorphism. The PCR condition for this gene was employed as follows. Denaturation at 95^0C for 5 min. followed by 30 cycles of 95^0C, 65^0C, 72^0C for 30 seconds and final extension of 72 ^{0}C for 5 min.

Statistical analysis

Chi-square analysis was utilized to test the frequencies of the genotype and alleles. Age,, occupation, smoking and drinking status were tabulated for cases and control groups. The association between polymorphism of *IL-10* -592 *A/C*, *IL-10*-1082 *G/A* polymorphism with the risk of prostate cancer was estimated by computing odds ratio (OR) and 95% confidence intervals (95% CI), using a multivariate logistic regression analysis that included several variables such as age, smoking, drinking, occupation, family history and stage of the disease. The

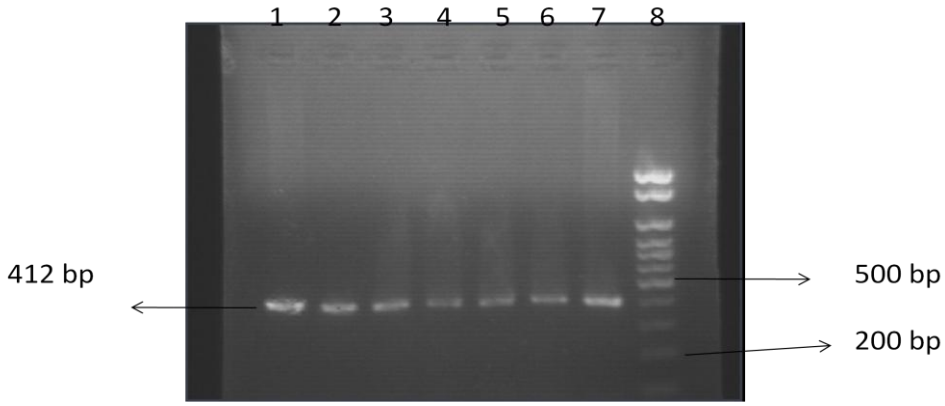

Figure 1. PCR amplified agarose gel picture of *IL-10*-592
Lane 1 412 bp pcr product
Lane 8 100 bp DNA markes

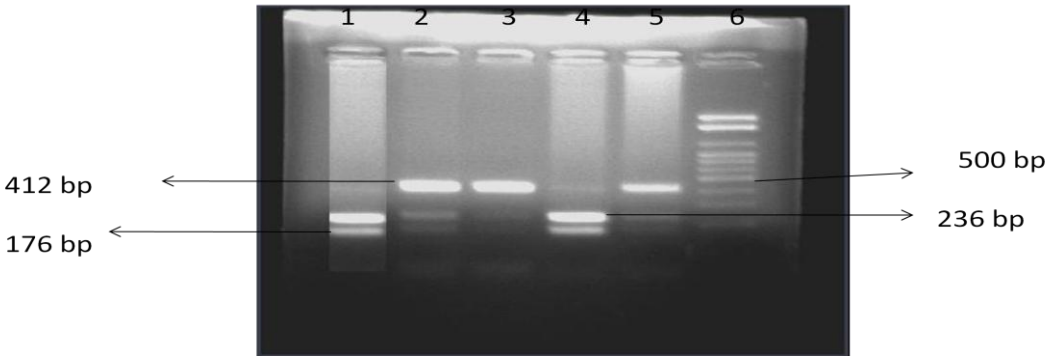

Figure 2. PCR RFLP (Rsa I) digest agarose gel reperesentative picture of IL-10-592
Lanes 1 and 4 236 and 176 bp *CC* genotype
Lanes 2 and 5 412, 236 and 176 bp *AC* genotype
Lane 3 412 bp *AA* genotype
Lane 6 100 bp DNA marker

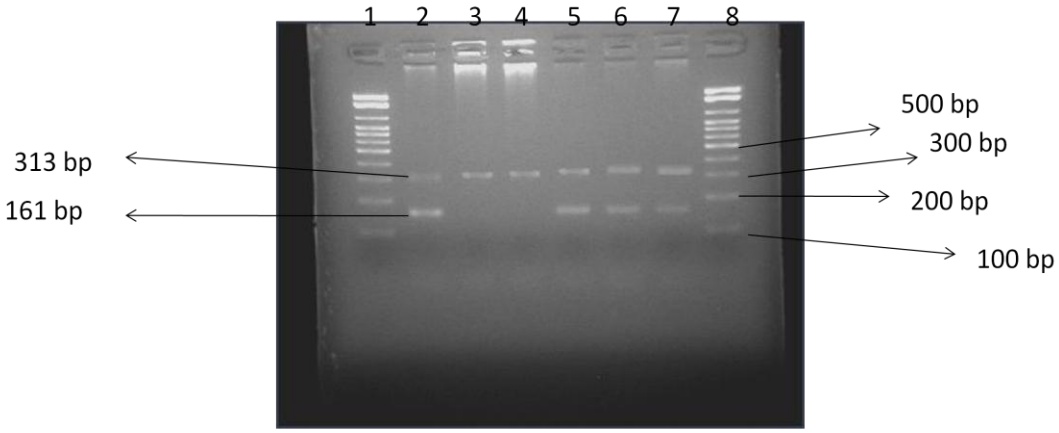

Figure 1. ARMS agarose gel representative figure of IL-10- 1082
Lanes 1 and 11 100 bp DNA ladder
(upper band 313 bp internal control)
Lanes 2 and 3 161 bp *GG*
Lanes 4 and 5 161 bp *AA*
Lanes 6, 7 161 bp *GA*

Table 1. *IL-10* -592 *A-C* genotype frequencies, OR and 95% CI for prostate cancer among cases and controls

Genotype	Cases	Conterols	OR (95% CI)
AA	63 (42.0%)	75 (50 %)	1.0 Ref.
AC	75 (50%)	67 (44.7%)	1.33 (0.81-2.19)
CC	12 (8%)	8(5.3%)	1.79 (0.63-5.15)
Allelic frequency			
A	201	217	1.0 Ref.
C	99	83	1.29 (0.89-1.85)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention).

Table 2. *IL-10* -592 *A-C* genotype frequencies and OR and 95% CI for prostate cancer among cases and BPH

Genotype	Cases	BPH	OR (95% CI)
AA	63 (42.0%)	71 (47.3%)	1.0 Ref.
AC	75 (50%)	69 (46.0%)	1.22 (0.74-2.02)
CC	12 (8%)	10 (6.7%)	1.35 (0.50-3.66)
Allelic frequency			
A	201	211	1.0 Ref.
C	99	89	1.17 (0.81-1.67)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention).

Table 3. Genotype distribution of *IL-10* -592 *A/C* polymorphism in comparison with smoking among prostate cancer patients and controls.

	Smokers			Non smokers		
Genotype	**cases**	**controls**	**OR (95% CI)**	**cases**	**controls**	**OR (95% CI)**
AA	24 (16%)	22 (14.7%)	1.23 (0.85-1.77)	39(ref.) (26%)	53(ref.) (35.3%)	1.0
AC	20 (13.3%)	15 (10%)	1.81 (0.77-4.29)	55 (36.7%)	52 (34.7%)	0.85 (0.39-1.82)
CC	4 (2.7)	3 (2%)	1.35 (0.68-2.67)	8 (5.3%)	5 (3.3%)	1.43 (0.64-3.18)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention)

Table 4. Genotype distribution of *IL-10* -592 *A/C* polymorphism in comparison with smoking among prostate cancer patients and BPH.

	Smokers			Non smokers		
Genotype	**cases**	**BPH**	**OR (95% CI)**	**cases**	**BPH**	**OR (95% CI)**
AA	24 (16%)	16 (10.7%)	1.16 (0.79-1.68)	39 ((ref.) 26%)	55 (ref.) (36.7%)	1.0
AC	20 (13.3%)	18 (12%)	1.57 (0.69-3.58)	55 (36.7%)	51(34%)	0.87 (0.40-1.86)
CC	4 (2.6)	2 (1.3%)	1.61 (0.87-2.99)	8 (5.3%)	8 (5.3%)	1.07 (0.45-2.54)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention)

statistical analysis was performed using Epi-Info software (Epi-Info, version 3.5.1).

Written informed consent was obtained from all cases and controls. The study was carried out after obtaining approval from the Ethics Committee of the Postgraduate Institute of Medical Education and Research, Chandigarh, India.

RESULTS

Demographic characteristics of study subjects

The mean age among cases, controls and BPH study subjects was 67.49±7.92, 65.57± 8.21 and 66.71±9.24, respectively. *p* value was greater than 0.05 for both cases, BPH and healthy controls. Among cases 56.7% were from rural and the remaining 43.3% were from urban area. In the BPH study subjects 56% were from urban as compared to the remaining 44% from rural area. On the other hand, 57.3% of controls were from rural as opposed to 42.7% from urban area. Regarding occupation 46% of cases were sedentary workers, 50.7% were manual and the rest 3.3 % were industrial workers. In BPH, study subjects sedentary workers were 45.3%, manual 52% and 2.7% were industrial workers. When the percentage of occupation among controls was analyzed sedentary, manual and industrial workers were 48, 49.3 and 2.7%, respectively. Regarding smoking status there were 102 (68%) non smokers within cases, 114 (76%) BPH and 110 (73.3%)

Table 5. *IL-10* 1082 *A-G* genotype frequencies and OR and 95% CI for prostate cancer among cases and controls

Genotype	Cases	Controls	OR (95% CI)
AA	50 (33.3%)	47 (31.3%)	1.0
AG	73 (48.0%)	75 (50.0%)	0.91 (0.53-1.58)
GG	27 (18.0%)	28 (18.7%)	0.91 (0.44-1.85)
Allelic frequency			
A	173	169	1.0
G	127	131	0.95 (0.68-1.33)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (centre for disease control and prevention)

Table 6. *IL-10* 1082 *A-G* genotype frequencies and OR and 95% CI for prostate cancer among cases and BPH

Genotype	Cases	BPH	OR (95% CI)
AA	50 (33.3%)	45 (30%)	1.0 Ref.
AG	73 (48.0%)	79 (52.7)	0.83 (0.48-1.44)
GG	27 (18.0%)	26 (17.3%)	0.93 (0.70-1.34)
Allelic frequency			
A	173	169	1.0 Ref.
G	127	131	0.95 (0.68-1.33)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (center for disease control and prevention).

Table 7. Genotype distribution of *IL-10* -1082 *A-G* polymorphism in comparison with smoking among prostate cancer patients and controls.

	Smokers			Non smokers		
Genotype	**cases**	**controls**	**OR (95% CI)**	**cases**	**controls**	**OR (95% CI)**
AA	15 (10%)	13 (8.7%)	1.12 (0.43-2.96)	35 (ref.) (23.3%)	34 (ref.) (22.7%)	1.0 Ref.
AG	26 (17.3%)	19 (12.6)	1.33 (0.58-3.04)	47 (31.4%)	56 (37.4)	1.05 (0.46-2.43)
GG	7 (4.6%)	8 (5.3%)	0.85 (0.24-2.96)	20 (13.4)	20 (13.4)	0.79 (0.24-2.55)

OR= odds ratio, CI= Confidence interval, OR was computed by Epi-Info version 3.5.1. (center for disease control and prevention)

Table 8. Odds ratio (OR) and corresponding 95% confidence interval (CI) for the combined effect of *IL-10*- 592 *A-C* and *IL-10* 1082 *A-G*

***IL-10* -592**	***IL-10* 1080**	**Cases (%)**	**Control (%)**	**BPH (%)**	**OR (95%CI)**
AA	*AA*	21 (14)	27 (18)	25 (16.7)	1.0 Ref.
	AG	29 (19.3)	38 (25.3)	29 (19.3)	0.98 (0.43-2.22)
	GG	13 (8.7)	10 (6.7)	17 (11.3)	1.67 (0.55-5.14)
	AA	23 (15.3)	17 (11.3)	18 (12)	1.74 (0.69-4.44)
AC	*AG*	40 (26.7)	34 (22.7)	42 (28)	1.51 (0.68-3.36)
	GG	12 (8)	16 (10.7)	9 (6)	0.96 (0.34-2.74)
	AA	6 (4)	3 (2)	2 (1.3)	1.52 (0.87-2.67)
CC	*AG*	4 (2.7)	3 (2)	8 (5.3)	1.31 (0.64-2.68)
	GG	2 (1.3)	2 (1.3)	-	1.14 (0.41-3.20)

within controls. The remaining 32% in cases, 24% in BPH and 26 % in controls were smokers. Whenever the status of alcoholism was analyzed 58.7% of cases were non drunker as compared to 59.3% in controls and 58% in BPH. The rest 41.3% had reported drinking at least 3 times per week in cases and 40.7% and 41.3% had reported having similar drinking situation in controls and BPH, respectively.

Table 1 demonstrates the frequencies of the three genotypes of *IL-10*-592 *A-C* polymorphism among cases and healthy controls. The percentages of *AA*, *AC* and *CC* genotypes among cases were 42, 50 and 8, respectively. However, in BPH the percentage of *AA* genotype was 47.3 and while that of *AC* and *CC* were 46 and 6.7, respectively. Statistically non significant 1.29 folds of marginal increased risk to prostate cancer among cases was associated with

the *CC* genotype of *IL-10* -592 *A-C* gene (OR=1.29, 95%CI=0.89-1.85).

Table 2 describes the frequencies of the three genotypes of *IL-10*-592 *A/C* polymorphism among cases and BPH. The percentages of *AA*, *AC* and *CC* genotypes among cases were 42, 50 and 8, respectively. However, in BPH the percentage of *AA* genotype was 47.3 and while that of *AC* and *CC* were 46 and 6.7, respectively. Statistically non cancer among cases was associated with the *CC* genotype of *IL-10* -592 *A-C* gene (OR=1.35, 95%CI=0.50-3.66). significant 1.35 folds of marginal increased risk to prostate

The relative risk of smoking in association with *IL-10*-592 *A/C* polymorphism among cases and controls is given in Table 3. The percentages of the three genotypes of *IL-10* -592 *A/C* polymorphism in smoker cases were *AA* 16, *AC* 13.3 and *CC* 2.7, respectively. The percentages of the three genotypes in smoker controls were 14.7, 10 and 2 for *AA*, *AC* and *CC* genotypes, respectively. The association of smoking and *IL-10*- 592 *A/C* genotype of cases was computed against healthy non smoking controls. There was statistically non significant 1.81 folds of increased risk of prostate cancer to cases due to the association of smoking and *AC* genotype of *IL-10* -592 A/C genotype (OR=1.81, 95% CI=0.77-4.29).

The relative risk of prostate cancer in smoker cases along with *IL-10*-592 *C/A* polymorphism was computed against BPH non smoking study subjects in Table 4. The frequency of the three genotypes in smoker BPH study subjects were 10.7, 12, and 1 percent for *AA, AC* and *CC,* respectively. There was no statistically significant variation among cases and BPH when ever smoking was taken as a risk factor along with *IL- 10*-592 polymorphic forms. However, statistically non significant increased risk of prostate cancer was associated with *AC* and *CC* genotypes of *IL-10*-592 polymorphism in cases (OR= 1.57,95% CI= 0.69-3.58 and 1.67, 95 % CI 0.87-2.99, respectively).

Whenever the effect of alcohol along with polymorphism of *IL-10*-592 *A/C* towards overall risk of prostate cancer is computed, there were 19.3% *AA* genotype carriers from all alcoholic cases and the percentage of *AC* genotype in drunker cases was 18, the remaining 4% drunker had the *CC* genotype. On the hand, there were 22% *AA* genotype carriers among drunken controls and the percentage of *AC* and *CC* genotype within drunker controls were 16.7 and 2, respectively. The association of alcohol to the risk of prostate cancer in cases was computed against non alcoholic healthy controls. There was statistically non significant association of alcohol and alcohol and *CC* genotype *of IL-10*-592 in cases (OR= 1.49, 95 %CI= 0.88-2.52).

The percentage of the three genotypes of *IL-10* 1080 *A/G* genotype is given in Table 5. The frequency of *AA* genotype in cases was 33.3 percent and it was 31.3 percent in controls. The frequency of the heterozygous *AG* genotype in cases was 48 percent and it was 50 percent in controls. The percentage of the homozygous *GG* genotype was 18 percent in cases and it was 18.7 percent in controls. No statistically significant difference in the genotype frequency among cases and control was observed.

The frequency of *IL-10* 1082 *A/G* genotype polymorphism in cases was also computed against BPH study subjects (Table 6). The percentages of the three genotypes in BPH were *AA* 30, *AG* 52.7 and *GG* 17.3, respectively. When risk of prostate cancer in cases was analyzed against BPH study subjects, there was statistically non significant association for risk of prostate cancer among cases in regard to this gene polymorphism.

The effect of smoking, *Il-10 1082 A/G* polymorphism and the risk of prostate cancer is computed in Table 7. The percentage of *AA*, *AG* and *GG* genotypes in smoker cases were 10, 17.3 and 4.6, respectively. The percentages of the three genotypes in non smoker cases were 23.3, 31.4 and 13.4. The percentages of *AA*, *AG* and *GG* genotypes in smoker controls were 8.7, 12.6 and 5.3, respectively. The OR for smoking and this gene polymorphism for cases were computed against healthy non smoker controls. There was no statistically significant association between smoking, this gene polymorphism and risk of prostate cancer.

The gene-gene interaction of *IL-10*-592 *A/C* and *IL-10*-1082 *A/ G* is given in Table 8. There was statistically no significant association between any of these genotype combinations and risk of prostate cancer in cases. However, statistically non significant 1.74 and 1.67 folds of increased risk of prostate cancer was observed due to the gene-gene interactions of *AC* of *IL-10*-592, d *GG* of *IL-10* 1082 and *AA* of *IL-10* -592, *CC* of *IL-10* 1082, respectively.

DISCUSSIONS

Interleukin (*IL-10*) is considered as a key mediator of immunosuppressant and tolerance. It appears to be primarily produced by monocytes and T regulatory (Tr)1-lymphocytes and to act on a number of cells, including monocytes, helper and cytotoxic T cells as well as B cells. IL-10 was shown to play a role in a broad variety of tumors, autoimmune and infectious diseases (Moore et al., 2001). Evidence for its immunosuppressive activity has been based on the observations that (i) increased production of IL-10 *in vivo* and in animal models was associated with states of Immunosuppression (Mahanty et al., 1996; Plebanski et al., 1999) (ii) neutralizing antibodies to IL- 10 reversed hyporesponsiveness of T cells *in vivo*, (King et al., 1993 ; Osborne and Devaney 1999) and (iii) deletion of the *IL-10* gene rendered mice more resistant to tumors or infection and more susceptible to infection pathology or autoimmune disease (Kuhn et al., 1993; Hoffmann et al., 2001).

The promoter polymorphisms of *IL-10* have been subject to the most scrutiny, particularly with regard to possible influences upon gene transcription and protein production. For example, the *IL-10* -1082 SNP and -1082, -819, -592 haplotype have been reported to be associated with differential IL-10 expression *in vitro*, with the -1082 *A,* -819 *T,*-592 *A* haplotype associated with decreased IL-10 expression, compared with the -1082 *G*, -819 *C*, -592 *C* haplotype (Howell and Rose-Zerilli, 2006). It has been proposed that IL-10 might contribute to the tumorigenesis of various types of cancers such as lymphoma (Levy and Brouet, 1994) colon (Gastl et al., 1993), and ovarian (Pisa et al., 1992). Moreover, Kozlowski and co-workers (2003) reported higher amount of IL-10 in breast cancer patients compared to healthy women, and also a correlation between IL-10 levels and clinical stages of the disease (Kozlowski et al., 2003). However, anti-tumor effects of IL-10 via mechanisms such as inhibition of angiogenesis and enhancing anti-tumor activity of natural killer cells have also been described (Kundu and Fulton 1997; Sturlan et al., 2001).

In the present study IL-10-592 A/C and *Il-101082 A/G* polymorphisms were analyzed to see its role in risk of prostate cancer in north Indian study subjects. No statistically significant association for risk of prostate cancer was observed to any of the genotypes of IL-10-592 polymorphism. However, statistically non significant slight increased risk of prostate cancer was associated between the *CC* genotype of cases (OR= OR=1.79, 95% CI=0.63-5.15). This non significant association could be explained in that, *IL10* –592*CC* genotype has been related to a low IL-10 production capacity (Temple et al., 2003) trough this balance between pro and anti inflammatory situation may interplay leading in to more inflamination and occurrence of cancer. Our result is consistent with four studies that examined variation in *IL-10* loci and association with prostate cancer incidence and/or tumor characteristics. Three of these studies found no association. Eder and colleagues (2007) examined the -592 *IL-10* SNP and found no association with the development of prostate cancer (Sturlan et al., 2001). Xu *et al*. (2005) also did not find an association between the *IL-10* SNPs at -1082 and -592 and prostate cancer incidence (Eder et al., 2007). Similar to our study the study by Eder *et al*. (2007) and Xu *et al*. (2005) showed the minor allele frequencies at these loci did not differ between cases and controls (Sturlan et al., 2001 ;Eder et al., 2007). Moreover, Michaud and colleagues (2006) examined genetic variation in three *IL-10* loci in subjects from the United States participating in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial and did not find an association of genotype at any of the loci with prostate Cancer (Xu et al., 2005). However, Faupel- Badger *et al.* (2008) reported that homozygous variant alleles at either -819 or -592 in the *IL-10* promoter are associated with increased risk of prostate cancer among American population (Michaud et al., 2006).

The difference between our results and this report may be due to sampling or to inherited genetic differences between these two populations. Moreover, Faupel- Badger and colleagues have examined three polymorphic loci at the promoter region of *IL-10*, however, in this study only two loci has been studied due to unavailable circumstances. While a large number of investigations of possible associations between *IL-10* genotypes and immune mediated disease have been performed, the literature with regard to *IL-10* polymorphisms and cancer is as yet small, but growing (Michaud et al., 2006).

Whenever risk factors like smoking and alcoholism were computed we did not find significant association with risk of prostate cancer in cases this may be most likely due to less power of the statistics as number of smokers and alcoholic cases reported in this study were very small.

Interleukin-10 (*IL-10*) is an anti-inflammatory cytokine, which is involved in down- regulating cell-mediated and cytotoxic inflammatory responses (Faupel-Badger et al., 2008). *IL-10* polymorphism has been extensively studied and reported to be associated with other cancers and diseases. A case-control study including 500 female patients with histological confirmed breast cancer and 500 female, age matched, healthy control subjects conducted by Langsenlehner and colleagues suggested that *IL-10* promoter polymorphism was associated with decreased breast cancer risk (Zhou et al., 2008). A prospective, case-control study including 147 patients with advanced ovarian cancer conducted by Ioana and colleagues showed that *IL-10* promoter polymorphism may be related with the ability to achieve optimal tumour debulking, and it seemed to influence the overall and disease-free survival rate (Langsenlehner et al., 2005). In this study, where polymorphisms of *IL-10*-1082 *G-A* was analyzed to see its effect as risk factor for PCa, there was no statistically significant association for risk of PCa and this gene polymorphism in north Indian population.

*IL-10-G*1082*A* polymorphism is correlated with the expression of IL-10 and accordingly affects the susceptibility to some types of tumors, such as cervical cancer (Ioana Braicu et al., 2007) and prostate cancer (Roh et al., 2002). Although the expression of IL-10 gene might be associated with the susceptibility to esophageal scoumous cell carcinoma (McCarron et al., 2002), the correlation of IL-10 -1082 promoter polymorphism with the risk of prostate cancer in north Indian population has not been reported so far. Some workers have suggested that as much as 75% of inter-individual variation in IL-10 expression may be due to genetic variation, although others believe that the contribution of individual SNPs – such as the best-described -1082 SNP – may be much less than this. However, at this stage the precise role of *IL-10* promoter polymorphisms – both individually and as part of defined haplotypes and mosaics in determining IL-10 transcription and expression levels is still a subject under active investigation (Howell and Rose-Zerilli, 2006).

Whenever life style risks like smoking and alcoholism were analyzed to determine their role in prostate cancer risk, no statistically significant association was observed between these factors and prostate cancer. Another study with large sample size with same and different ethnic backgrounds is warranted to exactly determine the association between this polymorphism, lifestyle and risk of prostate cancer.

While the precise mechanisms by which *IL-10* polymorphisms may modulate PCa progression remains known, evidence suggests that IL-10 modulates immune function, such as NK cell, T cells, and macrophages activity, which would alter disease progression. Additionally, increasing evidence suggests a role for IL-10 inhibition of angiogenesis, therefore decreases IL-10 expression would depress angiogenic activity and promote cancer progression (Nagata et al., 2002). The non significant association of increased PCa risk duo to the gene- gene interaction of *AC* of *IL-10*-592 and *GG* of *IL-10* 1082 indicated in this study might be due to the genotypic interplay of the two genes which may decrease the secretion of IL-10 that enhances inflaminaion through indirect role. However, this needs to be confirmed *in virto* and *in vivo* studies and thus, other similar studies in the same and different population with increase sample size is recommended.

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